



**UNIVERSITI PUTRA MALAYSIA**

**ENZYMATIC SYNTHESIS OF PALM BASED  
AMINO ACID ESTERS**

**SOO EE LIN**

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**ENZYMATIC SYNTHESIS OF PALM BASED  
AMINO ACID ESTERS**

**By**

**SOO EE LIN**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in  
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**Chairman: Professor Abu Bakar Salleh, Ph.D.**

**Faculty: Science and Environmental Studies**

Acyllysines were successfully prepared from reactions of free fatty acids (FFAs) (palmitic acid, PA; oleic acid, OA), triglycerides (TGs) (tripalmitin, TP; triolein, TO) and palm oil fractions (palm olein, PO; palm kernel olein, PKO) with the unprotected amino acid, L-lysine, using enzymes as biocatalyst in an organic solvent system. Such a selective enzymatic route obviates the fastidious regime of substrate activation/protection required in chemical synthesis. Preliminary detection and identification of reaction products were facilitated by thin layer chromatography (TLC) while subsequent quantitative studies on main and interactive effects of parameters governing the reactions were conducted with TLC-photodensitometry.

Enzyme screening revealed Lipozyme IM to be the most efficient biocatalyst for the reactions. By varying one-parameter-at-a-time, 70°C was shown to be the optimal temperature, hexane, the most suitable solvent, and, 5:1 (PA:L-lysine), 3:1 (OA:L-

lysine), 1:1 (TP:L-lysine), 1:3 (TO:L-lysine), 1:1 (PO:L-lysine) and 1:1(PKO:L-lysine) the respective optimal substrate molar ratios. To reach equilibrium, reactions of FFAs and TGs with L-lysine required 4 days while those of the more complex PO and PKO required 6 days. Higher biocatalyst amounts improved all reactions (except that of TP with L-lysine), as did higher amounts of molecular sieves added to reactions of OA and TO with L-lysine.

Response surface methodology (RSM) was then used to study interactive effects of the parameters. Best fitting models for reactions of FFAs and TGs with L-lysine were third order polynomials while those for palm oil fractions were second order polynomials. Generally, simultaneously increasing temperature and solvent hydrophobicity, fatty substrate concentration, enzyme amount or molecular sieves amount improved yields except for the PKO reaction where elevated temperatures compromised yields irrespective of enzyme and desiccant amount. Increasing solvent hydrophobicity and fatty substrate concentration or enzyme amount synergistically improved reactions, as did increasing substrate concentration and enzyme amount. On the contrary, in reactions of OA, TP, PO and PKO with L-lysine, increasing desiccant amount in very non-polar solvents, at very high levels of enzyme, and, in very concentrated substrate solutions showed a slight negative effect.

Optimal conditions derived from RSM differed slightly from those of the conventional "one-variable-at-a-time" approach due to the latter's inability to consider interactions among the parameters. However, the order of preference in reactivity of acyl donors was very consistent in both studies in that PKO was the most efficient acyl donor followed

by PO, TO and OA whereas TP and PA displayed poorest performance. Reactions carried out at larger volumes of 150mL under RSM-optimised conditions yielded 34.3% (0.6585g), 4.4% (0.0893g), 14.7% (0.2823g), 1.6% (0.0324g), 3.9% (0.0747g) and 4.0% (0.0776g) of products from the reactions of L-lysine with PA, OA, TP, TO, PO and PKO, respectively, after purification by column chromatography. Identities of isolated products were verified by comparison to commercial standards using TLC, gas chromatography (GC) and infrared spectroscopy (IR).

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**SINTESIS ESTER ASID AMINO BERASASKAN  
MINYAK KELAPA SAWIT MENGGUNAKAN ENZIM SEBAGAI MANGKIN**

**Oleh**

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Asillisin berjaya dihasilkan melalui tindakbalas di antara asid lemak bebas (asid palmitik, PA; asid oleik, OA), trigliserida (tripalmitin, TP; triolein, TO) dan fraksi minyak kelapa sawit (olein minyak kelapa sawit, PO; olein isirong kelapa sawit, PKO) dengan asid amino, L-lisin, menggunakan enzim sebagai pemangkin dalam sistem pelarut organik. Dengan kaedah pemangkinan berenzim yang selektif, tindakbalas dapat dicapai tanpa mengaktifkan substrat sebagaimana yang diperlukan melalui sintesis kimia. Pengesanan dan identifikasi produk dijalankan dengan cara kromatografi lapisan nipis (TLC). Seterusnya, TLC-fotodensitometri digunakan dalam analisis kuantitatif untuk mengkaji kesan parameter-parameter atas tindakbalas antara substrat-substrat tersebut.

Antara beberapa jenis enzim yang dikaji, lipase sekat-gerak Lipozyme IM didapati memberikan hasil terbaik. Dengan menukarkan parameter tindakbalas enzim satu demi satu, didapati hasil optima dicapai pada suhu 70°C, dengan kehadiran heksana sebagai

pelarut organik, dan, menggunakan nisbah substrat berlemak:L-lisin 5:1 (PA:L-lisin), 3:1 (OA: :L-lisin), 1:1 (TP:L-lisin), 1:3 (TO:L-lisin), 1:1 (PO:L-lisin) and 1:1 (PKO:L-lisin). Untuk mencapai keseimbangan, tindakbalas antara asid lemak bebas dengan trigliserida dengan L-lisin mengambil masa 4 hari manakala tindakbalas antara kedua-dua fraksi minyak kelapa sawit dengan L-lisin memerlukan 6 hari. Hasil didapati meningkat apabila kuantiti enzim dipertingkatkan kecuali dalam tindakbalas antara TP dengan L-lisin. Hasil juga didapati meningkat dengan penambahan agen pengering kepada tindakbalas OA dan TO dengan L-lisin.

Seterusnya, kaedah permukaan respon (RSM) digunakan untuk mengkaji interaksi antara kesan-kesan parameter atas tindakbalas tersebut. Tindakbalas antara asid lemak bebas dan trigliserida dengan L-lisin sesuai dimodelkan dengan polinomial tertib ketiga manakala tindakbalas antara PO dan PKO dengan L-lisin lebih sesuai dimodelkan dengan polinomial tertib kedua. Kenaikan suhu bersama peningkatan ciri hidrofobik pelarut organik, kepekatan substrat berlemak, kuantiti enzim dan kuantiti agen pengering didapati meningkatkan perolehan asillisin kecuali dalam tindakbalas PKO di mana peningkatan suhu menurunkan hasil tanpa dipengaruhi kuantiti enzim dan agen pengering. Peningkatan ciri hidrofobik pelarut organik bersama peningkatan kepekatan substrat berlemak atau kuantiti enzim, dan, peningkatan kepekatan substrat berlemak bersama kuantiti enzim juga meningkatkan hasil tindakbalas. Dalam tindakbalas antara OA, TP, PO dan PKO dengan L-lisin, peningkatan kuantiti agen pengering dalam pelarut organik yang sangat hidrofobik dan, dengan menggunakan kuantiti enzim yang banyak dan larutan substrat yang pekat didapati memberi kesan negatif.

Bagi tindakbalas-tindakbalas tersebut, keadaan optimum yang dirumuskan melalui kaedah RSM didapati berlainan daripada keadaan optima yang dihuraikan melalui kaedah lama "mengganti-parameter-satu-demi-satu". Perbezaan ini berpunca dari kegagalan kaedah lama untuk mempertimbangkan interaksi-interaksi antara parameter-parameter ujikaji. Walaubagaimanapun, aturan reaktiviti substrat berlemak didapati sama, apabila menggunakan kedua kaedah kajian, yang mana PKO didapati sangat reaktif diikuti PO, TO dan OA manakala TP dan PA menunjukkan reaktiviti yang rendah. Eksperimen pada skala lebih besar (150mL) yang dijalankan dalam keadaan optima berdasarkan RSM telah menghasilkan 34.3% (0.6585g), 4.4% (0.0893g), 14.7% (0.2823g), 1.6% (0.0324g), 3.9% (0.0747g) dan 4.0% (0.0776g) produk dari tindakbalas antara L-lisin dengan PA, OA, TP, TO, PO dan PKO, masing-masing, selepas pengasingan dan penulenan dengan kromatografi turus. Identiti produk-produk tersebut disahkan melalui perbandingan kepada standard komersial dengan menggunakan teknik-teknik TLC, kromatografi gas (GC) dan spektroskopik inframerah (IR).



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I certify that an Examination Committee met on 24<sup>th</sup> April, 2003 to conduct the final examination of Soo Ee Lin on her Doctor of Philosophy thesis entitled "Enzymatic Synthesis of Palm Based Amino Acid Esters" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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## DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

  
SOO EE LIN

Date: 26/06/03

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## LIST OF ABBREVIATIONS

BOP	benzotriazole-1-yl-oxy-tris(dimethylamino)phosphonium hexafluorophosphate
CaCl <sub>2</sub>	calcium chloride
CCRD	central composite rotatable design
CMC	critical micelle concentration
d	day
DHA	docosahexaenoic acid
EPA	eicosapentaenoic acid
E/S	enzyme/substrate ratio
FFA	free fatty acid
F-ratio	Fischer variance ratio
GC	gas chromatography
HMDS	hexamethyldisilazane
HCl	hydrochloric acid
h	hour
IR	infrared
mM	millimolar
mpt	melting point
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> ·10H <sub>2</sub> O	<i>di</i> -sodium tetraborate decahydrate
NaCl	sodium chloride
Na <sub>2</sub> HPO <sub>4</sub>	<i>di</i> -sodium hydrogen orthophosphate
NaOH	sodium hydroxide
NaSO <sub>3</sub>	sodium sulfite